IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2663
Steven M. RUBEN	Art Unit: 1644
Appl. No.: 10/662,429	Examiner: HUYNH, PHUONG N.
Filed: September 16, 2003	Atty. Docket: 1488.1890003/EJH/SAC
For: **Apoptosis Inducing Molecule I**	

Declaration of Markus Buergin
Ruben Exhibit #54

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Paper No. _____

Filed on Behalf of Party Ruben
By: Samuel B. Abrams, Esq.
Margaret B. Brivanlou, Esq.
JONES DAY
222 East 41st Street
New York, NY 10017
Tel: (212) 326-3939
Fax: (212) 755-7306

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES
(Administrative Patent Judge Sally Gardner Lane)

STEVEN M. RUBEN

Junior Party,
(Application 08/816,981),

v.

STEVEN R. WILEY and RAYMOND G. GOODWIN

Senior Party,
(Patent No. 5,763,223).

Patent Interference No. 105,077

DECLARATION OF MARKUS BUERGIN

Ruben EXHIBIT 2054
Ruben v. Wiley et al.
Interference No. 105,077
RX 2054

DECLARATION OF MARKUS BUERGIN

I, MARKUS BUERGIN, declare and state as follows:

1. Since June 1994, I have been employed by Human Genome Sciences, Inc. (HGS). From 1994 to 1998, I was a Research Associate in the Protein Expression and Purification Department. From 1998 to 2002, I was a Research Associate in the Protein Development, Expression Systems and High Throughput Screening Group. From 2002 to 2003, I was a Research Associate in the Protein Development, Analytical Group. Since 2003, I have been a Research Associate in the Pharmaceutical Science Department.

2. I have been asked by patent counsel for HGS to describe work I carried out related to a TNF ligand family member during the time from June 29, 1995 to March 14, 1996. In summary, I carried out an experiment to produce stably-transfected cell lines for each of two recombinant constructs encoding TNF delta (HLTBT71), a TNF ligand family member, which had been made by Guo-Liang Yu, then a scientist at HGS who was studying TNF delta, for the purpose of producing recombinant TNF delta for further analysis of the protein's biological properties. At the time, I was referring to this TNF delta protein encoded by HLTBT71 as TNFγ.

3. This procedure of producing the stable recombinant cell lines was a continuous, uninterrupted process of amplifying the TNF delta constructs from the time of transfection on November 29, 1995, through March 14, 1996 (and continuing thereafter). The amplification process entailed a standard, commonly used series of passages of the transfected cells into fresh media containing specific concentrations of methotrexate (MTX) which exert a selective pressure for resistance through gene amplification and are increased at successive points throughout the process. At various points in the process of passaging, an aliquot of cells was frozen and preserved as backup stocks, while the remaining cells continued to be cultured under

MTX selection, and advanced through the passaging process. Thus, for the samples of each clone that were not frozen away for backup stocks, the process was carried out continuously throughout the November 29, 1995 to March 14, 1996 time period.

4. I recorded the details of this continuous process of selecting stable TNF delta-expressing transfectants in my HGS laboratory Notebook Number 443 (copies of relevant pages of which are submitted as RE55), and are described below.

- On November 28, 1995, I cultured the dhfr- CHO host cells in 6 well dishes and incubated the cells overnight (RE55, page 20). On November 29, 1995, I removed the medium, washed the cells, and added transfection medium to the cells and I transfected the CHO dhfr- host cells with two different expression constructs of TNF delta (then called "TNFγ") (RE55, page 20). One expression vector was called N346, and the other was called CHOI (RE55, page 20). On December 1, 1995, the cells were seeded in hybridoma plates on selection medium containing either 20nM MTX or 100nM MTX and cultured under selection until January 3, 1996 RE55 pages 21-22 and 37).
- On January 3, 1996, I observed and recorded the condition of the cells cultured since December 1, 1995, picked 30 colonies of cells, and transferred them to fresh media containing 20nM MTX in 24 well dishes. The CHOI transfectant clones were labeled labeled 1.1-1.10, 2.1-2.9, and 3.1-3.11; and the N346 transfectant clones were labeled 1.1-1.2, 2.1-2.10, and 3.1-3.11. On January 9, 1996, I observed the condition of the colonies picked and cultured on January 3, 1996. (RE55 . pages 37-39)
- On January 15, 1996, I was out sick, but had a colleague of mine, Irina Knyazev, a fellow Research Associate at HGS, passage CHOI clone 2.7 from a T25 flask into a

T75 flask (RE55, page 40).

- On January 16, 1996, I passaged CHOI transfectant lines 1.2, 1.3, into a new flask, and 1.10, 2.3, 2.9, 1.6, 1.7, and 1.10 into new 6 well dishes (RE55 page 41).
- On January 17, I picked several new TNF delta transfectants that had been cultured in the hybridoma plates since December 1, 1995, labeled them clones 4.1-4.4, 5.1-5.10, and 6.1-6.10 (CHOI) and 4.1-4.10 and 5.1-5.4 (N346), and seeded them into 24 well plates containing 20 nM MTX (RE55, page 42).
- On January 22, 1996, I passaged CHOI clones 1.3, 1.2, 2.7, 1.6, 1.7, 1.9 (all fourth passage, *i.e.* "P04") and 2.3 and 2.9 (all third passage, *i.e.* "P03") into new vessels and media containing varying concentrations of MTX (0, 20, 50, 100, 200, 500 nM) for amplification (RE55i, page 44)
- On January 25, 1996, I passaged CHOI clones 1.3, 1.2, 2.7, 1.7, and 1.9 (all fifth passage, *i.e.* "P05") into new vessels and media for continued selection in varying concentrations of MTX and froze away an aliquot of each for backup storage (RE55, pages 45-46).
- On January 26, 1996, I passaged CHOI clones 2.3, 2.9, and 1.6 a fourth time; CHOI clones 3.3 and 1.10 a third time; CHOI clones 4.1-4.4, 5.5, 5.7, 5.9, 6.2, 6.10, and 6.6 a second time, and N346 clones 2.3, 4.1, 4.3-4.7, 4.10, 5.3, and 5.4 a second time RE55 , pages 46-48)
- On January 27, 1996, I replaced the culture medium of CHOI clones 1.2, 1.3, 1.7, 1.9, and 2.7 at passage P05 with serum free medium. The serum free medium was conditioned for use in Western blots and antiviral assays of Dan Bednarik, then a scientist at HGS (RE55 page 48).

- On January 29, 1996, I harvested five 1 mL aliquots of supernatant from each of the cell cultures of January 27, 1996. I froze three of these samples for Western analysis. The remaining two aliquots were provided to Dan Bednarik for antiviral assays (RE55i, page 48).
- On January 30, 1996, I passaged CHOI clones 1.6, 2.3, 2.9, 6.4, 6.7, and 6.8 a third time into varying concentrations of MTX (RE55, page 49).
- On January 30, 1996, I passaged CHOI clones 1.2, 1.3, 1.7, 1.9, and 2.7 from the fifth passage a sixth time into 20nM MTX. I also passaged CHOI clones 3.3, 1.10, 6.2, 6.6, and 6.10 into 20nM MTX to expand the cells for freezing (RE55 , page 50).
- On January 31, 1996, I harvested conditioned media of CHOI clones 1.6, 2.3, 2.9, 6.4, 6.7, and 6.8 for testing protein in Westerns and antiviral assays and incubated the cells for two more days (RE55 page 51). Also on January 31, 1996, I passaged CHOI clones 1.2, 1.3, 1.7, 1.9, and 2.7 from the fifth passage a sixth time into varying concentrations of MTX for continued amplification (RE55, page 52).
- On February 1, 1996, I passaged CHOI clones 1.6, 6.4, 6.7, and 6.8 from the third passage a fourth time into 20nM MTX to expand the cells for freezing (RE55 page 53). I also passaged N346 clones 4.2, 4.8, 4.9, 5.1, and 5.2 a second time into 20nM MTX; picked new N346 clones 6.1-6.10 from the hybridoma plates into for a first passage into 20nM MTX; and passaged CHO clones 5.1-5.4, 5.6, 5.10, 6.1, 6.5, 4.3, and 5.7, as well as 4.1, 4.2, 4.4, 5.5, and 5.9 into 20nM MTX to expand the cells for freezing (RE55i, pages 54-55).
- On February 2, 1996, I prepared and froze away backup stocks of CHOI clones 2.3, 2.9, 3.3, 6.2, 6.6, and 6.10; and I harvested and froze away CHOI clones 1.6, 2.3, 2.9,

6.4, 6.7, and 6.8 that had been incubating for two days since January 31, 1995 for testing protein in Westerns and antiviral assays (RE55, pages 55-56).

- On February 5, 1996, I prepared and froze away backup stocks of CHOI clones 1.10, 5.5, and 5.9 (RE55, page 57).
- On February 6, 1996, I passaged CHOI clones 6.5, 4.3, 5.7, 5.4, 4.1, 4.2, and 4.4 into 20nM MTX to expand the cells for freezing. I also passaged CHOI clones 1.2, 1.3, 1.7, 1.9, and 2.7 a seventh time, CHOI clones 1.6, 2.3, and 2.9 a sixth time, and CHOI clones 6.4, 6.7, and 6.8 a fourth time into varying concentrations of MTX for continued amplification (RE55 pages 58-59)
- On February 7, 1996, I submitted CHOI clones 1.6, 2.3, 2.9, 6.4, 6.7, and 6.8 that had been harvested on February 2, 1996 to Dan Bednarik for testing in antiviral assays (RE55 page 60).
- On February 8, 9, and 12, 1996, I prepared immunoprecipitated samples of TNF delta protein from CHOI clones 1.2, 1.3, 1.7, 1.9, 2.7, 1.6, 6.4, 6.7, 6.8, 2.3, and 2.9 for Western analysis, electrophoresed and blotted the samples and detected the TNF delta protein signal (RE55, pages 60-63, and 66-68).
- On February 9, 1996, I expanded CHOI clones 5.7, 5.4, 4.2, 6.5, and 4.1 in 20nM MTX to prepare them for freezing as backup stocks. I also passaged N346 clones 2.3, 4.1, 4.3-4.7, 4.10, 5.3, and 5.4 into varying concentrations of MTX for continued amplification as well as into 20nM MTX for freezing (RE55 pages 64-65).
- On February 13, 1996, I froze away backup stocks of N346 clones 2.3, 4.1, 4.3, 4.4-4.7, 4.10, 5.3, 5.4 and CHOI clones 4.3 and 4.4 (RE55 page 69).
- On February 14, 1996, I harvested N346 clones 2.3, 4.1, 4.3, 4.4-4.7, 4.10, 5.3, and

5.4 for testing in antiviral assays and Western blots. I also expanded N346 clones 4.2, 4.8, 4.9, 5.1, 5.2, and 6.1-1.10 in 20nM MTX to prepare them for freezing as backup stocks. (RE55, pages 70-71).

- On February 15, 1996, I continued the amplification of CHOI clones by passaging clones 1.2, 1.3, 1.7, 1.9, and 2.7 (an eighth time), clones 1.6, 2.3, and 2.9 (a seventh time), clone 6.8 (a fifth time), and clones 6.4 and 6.7 (a sixth time), as well as N346 clones 2.3, 4.1, 4.3, 4.4-4.7, 4.10, 5.3, and 5.4 (a fourth time) into varying concentrations of MTX (RE55 pages 71-72).
- On February 20, 1996, I expanded N346 clones 4.2, 4.8, 4.9, 5.1, 5.2, 6.1, 6.3, 6.4, and 6.6-6.10 into 20nM MTX to prepare them for freezing as backup stocks. I also continued the amplification of CHOI clones by passaging clones 1.2, 1.3, 1.7, 1.9, and 2.7 (a ninth time), clones 1.6, 2.3, and 2.9 (an eighth time), clone 6.8 (a sixth time), and clones 6.4 and 6.7 (a seventh time) into varying concentrations of MTX. (RE55. pages 73-75).
- On February 22, 1996, I performed an initial screening for high expressing clones of the CHOI and N346 TNF delta clones by Western analysis (RE55 pages 76-81).
- On February 24, 1996, I froze away backup stocks of N346 clones 6.8-6.10, 4.9, 4.8, 4.2, 6.7, 6.3, 6.6, 5.1, and 5.2; and continued the amplification of CHOI clones 2.3, 2.7, 1.3, 6.4, and 6.7 by passaging each yet again into varying concentrations of MTX (RE55 pages 83 and 84).
- On February 27 and February 29, 1996, I continued the amplification of CHOI clones 2.3, 2.7, 1.3, 6.4, and 6.7, as well as CHOI clones 1.6, 6.8, 1.2, and 1.9, by passaging each yet again into varying concentrations of MTX (RE55. pages 85-86).

- On March 1, 1996, I continued the amplification of N346 clones 2.3, 4.1, 4.3, 4.4, 4.5, 4.6, 4.7, 4.10, 5.3, and 5.4, by passaging each yet again into varying concentrations of MTX (RE55, pages 86-87).
- On March 1, 1996, I initiated a labeling study of several TNF delta clones, in which the cells were to be labeled with ^{35}S-cysteine and ^{35}S-methionine (RE55 page 87).
- On March 4, 1996, I seeded CHO TNF delta clones 2.7, 4.3, 6.4, and 6.7 into a six-well dish (RE55, page 88).
- On March 5, 1996, I continued the amplification of N346 clones 2.3, 4.1, 4.3, 4.4, 4.5, 4.6, 4.7, and 4.10, as well as CHOI clones 1.7, 6.8, and 2.9, by passaging each yet again into varying concentrations of MTX (RE55, page 89).
- On March 5, 1996, I added ^{35}S-cysteine and ^{35}S-methionine to each of the wells seeded with CHO TNF delta clones 2.7, 4.3, 6.4, and 6.7 on March 4, 1996 RE55 page 90).
- On March 6, 1996, I continued the amplification of CHOI clones 2.3, 2.7, 1.3, 6.4, and 6.7, as well as 1.6, 1.9, and 1.2, by passaging each yet again into varying concentrations of MTX. In addition, samples of each of these clones, except 2.3, were prepared for testing in an antiviral assay (RE55 pages 90-91).
- On March 11, 1996, I continued the amplification of CHOI clones 1.2, 1.6, 1.7, 1.9, 2.3, 2.7, 2.9, 1.3, 6.4, 6.7, and 6.8 by passaging each yet again into varying concentrations of MTX (RE55, page 96).
- On March 12, 1996, I continued the amplification of N346 clones 2.3, 4.1, 4.3, 4.4, 4.5, 4.6, 4.7, 4.10, 5.3, and 5.4 by passaging each yet again into varying concentrations of MTX. I also harvested samples of each of these N346 clones as

well as CHOI clones 1.2, 1.6, 1.9, 2.7, 1.3, 6.4, and 6.7 for testing in an antiviral assay. (RE55 5, pages 97-98).

- On March 14, I continued the amplification of CHOI clones 1.3, 6.4, 6.7, and 6.8 by passaging each clone yet again into varying concentrations of MTX (RE55 5, page 100).

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 22 Jun 04

Markus Buergin